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# Defensins at the Mucosal Surface: Latest Insights into Defensin-Virus Interactions

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**Defensins are innate immune effector peptides expressed at mucosal surfaces throughout the human body and are potently antiviral *in vitro*. The role of defensins in viral pathogenesis *in vivo* is poorly understood; however, recent studies have revealed that defensin-virus interactions *in vivo* are complicated and distinct from their proposed antiviral mechanisms *in vitro*. These findings highlight the need for additional research that connects defensin neutralization of viruses in cell culture to *in vivo* antiviral mechanisms.**

Human defensins are effector peptides produced by the innate immune system with broad antibacterial, antiviral, and antifungal activities (1). They have a conserved β-sheet fold and are divided into two families, α-defensins and β-defensins, based on cysteine bond formation and gene organization. Human β-defensins (HBD) are produced by skin and mucosal epithelial cells. Human α-defensins are further divided into two types; myeloid α-defensins (human neutrophil defensins 1 to 4 [HNP1–4]) are made by neutrophils and other myeloid cells, while enteric α-defensins (human defensins 5 and 6 [HD5–6]) are produced by Paneth cells in the small intestine (HD5 and HD6) or epithelial cells in the male and female reproductive tracts (HD5 only). Defensins are amphipathic and cationic, and both their antiviral and their antibacterial activity were originally attributed to lipid perturbations; however, the observation that several classes of nonenveloped viruses are also sensitive to defensins led to the discovery of additional antiviral mechanisms, which have been recently reviewed in detail (2). For enveloped viruses, disruption of viral glycoprotein functions involved in receptor binding and fusion predominate, particularly for HNP1–3, HD5, and HBD3, all of which are lectins. Additional mechanisms include receptor downregulation and disruption of early events in viral infection (e.g., reverse transcription of retroviruses). Although a universal mechanism for neutralization of nonenveloped viruses remains more elusive, some common themes have recently emerged in studies of human papillomavirus (HPV), human polyomaviruses, and human adenovirus (AdV). In most cases, α-defensin binding to the viral capsid allows entry but dramatically changes the intracellular trafficking of the incoming virus; however, these changes in intracellular trafficking are the result of virus-specific differences in proximal events (e.g., blocking uncoating or preventing a host-mediated cleavage event) (3–7). A shared mechanism for selective binding of α-defensins to nonenveloped viral capsids is suggested by the identification of an interface on one aspect of HD5 that governs antiviral activity against both human AdV and HPV, although the features on these disparate viral capsids that are recognized by α-defensins are not well defined (8–10). Despite progress in understanding the molecular interactions between defensins and viruses, the role of defensins in viral pathogenesis *in vivo* for both enveloped and nonenveloped viruses remains understudied; however, exciting recent advances have provided new insights (Fig. 1).

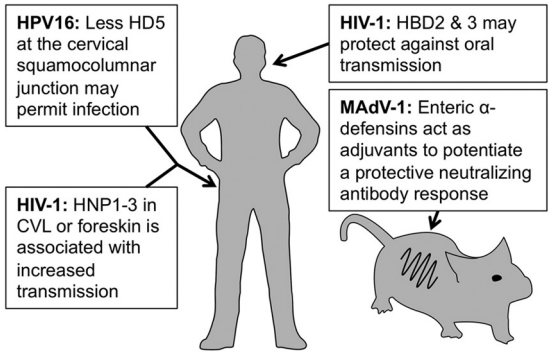


**FIG 1** Newly revealed roles for human defensins in viral infection and transmission. HPV16, human papillomavirus 16; HD5, human defensin 5; HIV-1, human immunodeficiency virus type 1; HNP1–3, human neutrophil peptides 1 through 3; CVL, cervical vaginal lavage fluid; HBD2, human β-defensin 2; MAdV-1, mouse adenovirus 1.

## α-DEFENSIN IMMUNOMODULATORY ACTIVITY MAY BE MORE IMPORTANT THAN DIRECT ANTIVIRAL ACTIVITY *IN VIVO*

α-Defensins are potently antiviral in cell culture, but do they have similar activities *in vivo*? In most cases, α-defensin concentrations *in vivo* can reach levels at which antiviral activity has been observed *in vitro* (2). Thus, direct antiviral activity *in vivo* is plausible. In the case of HPV, there has been interest in examining HD5 expression in cervical tissue, the site of HPV infection that leads to cancer (11). More than 90% of high-grade cervical intraepithelial neoplasias are thought to be due to HPV infection of a population of cells at the squamocolumnar junction, the region between the ectocervix and endocervix (12). HD5 is expressed in the ectocervix but not in the squamocolumnar junction or endocervix. It is possible that the absence of HD5 at this site allows for more HPV

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infection and therefore a greater possibility of cervical cancer progression (11). However, the squamocolumnar junction is also thought to be more permissive to HPV infection due to a surface-exposed population of stem cells (13). Therefore, to what extent the absence of HD5 at this location plays a role in HPV infection *in vivo* is unclear.

In the sole paper to directly test antiviral activity in a natural infection model, mice were challenged with mouse AdV type 1 (MAdV-1), which is sensitive to neutralization by mouse and human α-defensins in cell culture (14). In susceptible strains of mice, this mouse pathogen crosses the blood-brain barrier and causes fatal encephalitis (15). Upon infection by oral gavage, wild-type mice were significantly protected from MAdV-1 compared to mice lacking functional α-defensins due to deletion of the matrix metalloproteinase 7 gene (*Mmp7*$^{-/-}$), which is required for the activation of α-defensin precursors (14). The survival difference was maintained in mice depleted of commensal bacteria but absent upon parenteral infection, arguing for a specific effect of the α-defensins in the gut independent of their effects on the microbiota. A direct antiviral effect of α-defensins during the initial infection should delay or reduce viral dissemination from the gut to the brain; however, a time course quantifying viral genome copies in brain and spleen revealed equivalent dissemination kinetics in both mouse genotypes through day 9 postinfection. Only on day 11 were the viral loads in the brains and spleens of *Mmp7*$^{-/-}$ mice significantly higher than in wild-type mice, coincident with divergence of rates of survival and clinical presentations of *Mmp7*$^{-/-}$ and wild-type mice in survival studies. Thus, rather than having a direct antiviral-barrier role, the lack of α-defensins appeared to impact pathogenesis relatively late after infection. This is more consistent with an effect on the adaptive immune response to MAdV-1, an idea that was supported by histologic changes indicative of immune stimulation (e.g., germinal center formation and marginal zone thickening) in the spleens of wild-type mice but not *Mmp7*$^{-/-}$ mice. Moreover, neutralizing antibody titers, crucial for protection from MAdV-1 encephalitis, were reduced and delayed in the *Mmp7*$^{-/-}$ mice, although *Mmp7*$^{-/-}$ mice do not in general have an impaired humoral response. Thus, this first study to investigate the role of naturally secreted α-defensins in viral pathogenesis supports an adjuvant effect of the α-defensins rather than a direct antiviral effect at the site of initial infection. Similarly, β- and θ-defensins, whether naturally secreted or exogenously administered, have less of an effect as direct antivirals than by limiting immunopathology (16, 17). HNPs have been shown previously to function as adjuvants in mice (18, 19), but these prior studies did not include enteric α-defensins and did not demonstrate the functional consequences of defensin adjuvant activity during infection. This study raises a number of interesting questions. Is immune stimulation dependent upon α-defensin binding directly to the virus? Will antibody responses to other enteric viruses and other pathogens be similarly influenced by the presence of functional α-defensins? Will this response be dependent upon the effects of α-defensins on viral infectivity *in vitro*? Continued investigation along these lines will provide a crucial link between the known activities of α-defensins *in vitro* and their role in host defense *in vivo*.

## OPPOSING FUNCTIONS OF DEFENSINS IN HIV-1 INFECTION *IN VITRO* AND *IN VIVO*

Several recent papers have advanced our understanding of the role of defensins in human immunodeficiency virus type 1 (HIV-1) infection. Previous *in vitro* studies were consistent with the hypothesis that high levels of defensins present at mucosal sites may provide a first line of defense against HIV-1 by inhibiting HIV-1 infection through pleiotropic mechanisms that target cell binding, fusion, intracellular signaling, and gene expression (summarized in reference 2). Recent studies have confirmed the potency of HBD2 and HBD3 in blocking HIV-1 infection (20). Because these β-defensins are constitutively expressed at high levels in adult oral epithelium, they may play a more prominent role in contributing to the low levels of HIV-1 transmission in the oral cavity than other routes of transmission, as HBD2 and HBD3 expression in the anogenital mucosa is hormonally dependent (21). Consistent with this hypothesis, HBD2 and HBD3 bind to heparin sulfate proteoglycans on the surfaces of adult tonsil cells *in vitro* and cointernalize with HIV-1, eventually leading to neutralization in the endosomal compartment via unknown mechanisms (21). Interestingly, unlike in adults, in the fetal oral epithelium, HBD2 and HBD3 expression is low and remains low in infants for months after birth (22). Accordingly, HIV-1 transmigrated across both fetal and adult cells in an *in vitro* model of the oral epithelium, but only virions that passed through fetal cells or through cells where HBD expression had been knocked down via small interfering RNA (siRNA) were infectious (22). Thus, these studies support the notion that high levels of HBD2 and HBD3 expression are protective in the adult oral mucosa, and the low levels of these molecules may contribute to high rates of mother-to-child HIV-1 transmission in breastfeeding infants.

Studies of individuals at risk for HIV-1 infection and those who are HIV-1 exposed but remain seronegative (HESN) are a second line of investigation into the role of defensins in HIV-1 infection. Three recent studies assessed levels of defensins and other host defense molecules in cervical vaginal lavage fluid (CVL) of women and α-defensin and HIV-1-neutralizing IgA levels in the foreskin, the primary site of HIV-1 acquisition in heterosexual men (23–25). In CVL, levels of HNP1–3, HBD2, and HBD3 were all detectable (25). Genital infections, specifically with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, or *Candida* spp., were common in the subject population and associated with increases in one or more defensin levels. The ability of CVL depleted of IgA1 to broadly neutralize HIV-1 isolates from multiple clades was associated with higher HNP1–3 levels. Despite the capacity to neutralize HIV-1 *in vitro*, higher HNP1–3 levels were unexpectedly correlated with an increased risk of HIV-1 acquisition. Similarly, in men, although foreskin levels (swabs from the subpreputial space) of HNP1–3 but not HBD2 were significantly higher in HESN than in unexposed controls (24), a subsequent prospective study found that α-defensin levels were 10-fold higher prior to HIV-1 acquisition in patients who eventually acquired HIV-1 (23). Only HIV-neutralizing IgA was associated with protection. Therefore, when HIV-1 acquisition is used as an endpoint, high mucosal levels of α-defensin are repeatedly associated with HIV-1 infection. As HNPs inhibit HIV-1 infection *in vitro*, the mechanism of this increase in HIV-1 acquisition *in vivo* is not clear and warrants further study. It is possible that α-defensins directly enhance HIV-1 infection or that increased HIV-1 acquisition is due to the chemokine properties of α-defensins that

lead to increased target cell recruitment to mucosal sites, a complexity of α-defensin biology that uniquely impacts HIV-1 transmission. A final potential mechanism is that, as has been shown *in vitro*, α-defensins promote barrier permeability by disrupting tight junctions (26).

## CONCLUSIONS

Cell culture studies have demonstrated that α-defensins impact viral infection via a variety of mechanisms. While there are some similarities, the large number of antiviral mechanisms described to date highlights the specificity of the defensin-virus interaction. In the limited *in vivo* studies available, the role of α-defensins appears to be distinct from their potently antiviral activity *in vitro*. While immunomodulatory, adjuvant, and chemokine properties have long been ascribed to defensins, the importance of these functions *in vivo* highlights a need for more studies in these areas.

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